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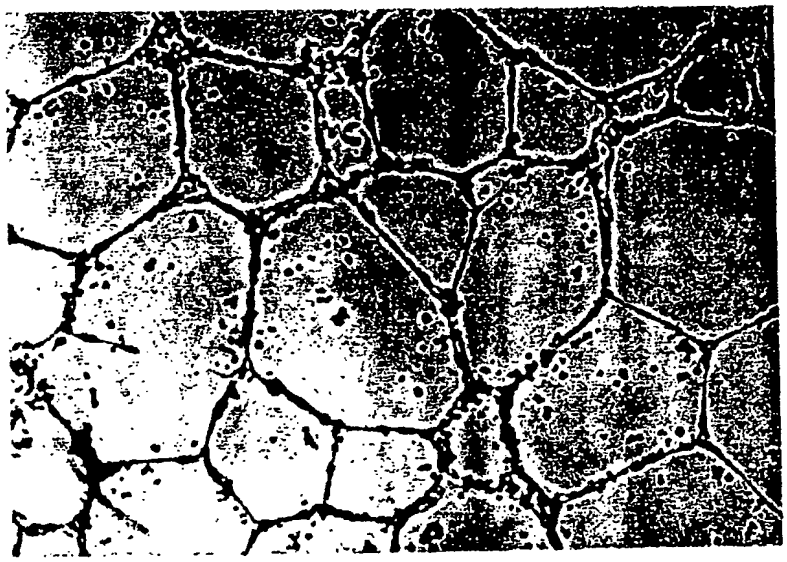

(57) Abstract: The present invention relates to a composition comprising Melissaleaf extract that inhibits angiogenesis and matrix metalloproteinase activity. The Melissa leaf extract of the present invention inhibits angiogenesis and activity of matrix metalloproteinase, sothat it can be applied to treat or prevent diseases related to angiogenesis and matrix metalloproteinase. The compositin of the present invention comprising Melissa leaf extract may also comprise more than one component of the other anti-angiogenic, anti-cancer, anti-inflammatory and anti-aging components. This particular composition comprising Melissa leaf extract can be used for pharmaceutical, dietetic and/or cosmetic purposes.

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COMPOSITION COMPRISING MELISSA LEAF EXTRACT FOR ANTI-ANGIOGENIC AND MATRIX METALLOPROTEINASE INHIBITORY ACTIVITY

BACKGROUND OF THE INVENTION

(a) Field of the Invention

The present invention relates to a composition comprising Melissa leaf extract having inhibitory activity on angiogenesis and matrix metalloproteinase.

In detail, the present invention relates to composition comprising Melissa leaf extract as active ingredient, which can be used as pharmaceuticals, food or cosmetics for treatment or prevention of angiogenesis- and/or MMP-dependent diseases.

(b) Description of the Related Art

Melissa (*Melissa officinalis*), a plant in a Labiatae family, is also called lemon balm, balm, or dropsy plant as common and folk names.

Key constituents of the *melissa officinalis* are volatile oils (geranial, neral, citronellal, linalool, geraniol, geranylacetate, methyl citronellate, trans-beta-ocimene, germacren, eugenol), caffeic acid derivatives, flavonoids, triterpenes, catechins and tannins. Rosmarinic acid, a derivative of caffeic acid, is the most abundant component (about 4.7%) of the Melissa leaf extract,

which is known to have anti-inflammatory activity.

Melissa is edible and medicinal. Fresh leaves can be added to salad and used to make sauces for fish, poultry and pork. Dried or fresh the whole plant is used to make cool refreshing drinks or warm relaxing teas, which is good for fevers, colds, and headache. As an alternative medicine, it is applied for calming nerves, relieving menstrual cramps, insomnia, depression, hyperthyroidism, upset stomach, and colic in babies. It is antibacterial, antispasmodic, antiviral, carminative, diaphoretic, digestive, emmenagogue, febrifuge, sedative, and tonic. Recently, Melissa leaf extract has included in blood circulation activator, which helps dilatation of peripheral blood vessels. Fresh crushed leaves are applied to wounds and insect bites. The oil from Melissa is often added to skin preparations and perfumes. The essential oil is also used in aromatherapy (Cohen RA, Kucera LS, Herrmann EC Jr., *Proc Soc Exp Biol Med* 117, 431-434,1964; Kucera LS, Cohen RA and Herrmann EC Jr, *Ann. NY Acad Sci* 130, 474-82, 1965).

Angiogenesis is the process of generating new capillary blood vessels. This is resulted from activated proliferation of pre-existing endothelial cells. Neovascularization is tightly regulated, and activation occurs only in embryogenic development, tissue remodeling, wound healing and periodic cycles of corpus luteum development (Folkman and Cotran, Relation of

vascular proliferation to tumor growth, *Int Rev Exp Pathol* 16 207-248, 1976).

The endothelial cells are growing very slowly as compared with other types of cells in the body. However, if the proliferation of these cells is induced by the failure of regulation of angiogenesis, some pathological status is developed (Timar, *J Pathol Oncol Res* 6, 85-94, 2001). Pathological angiogenesis is involved in many diseases. For example, cardiovascular diseases such as angioma, angiofibroma, vascular deformity, atherosclerosis, synechia and edemic sclerosis; and opthalmological diseases such as neovascularization after cornea implantation, neovascular glaucoma, diabetic retinopathy, angiogenic corneal disease, macular degeneration, pterygium, retinal degeneration, retrolental fibroplasias, and granular conjunctivitis are related to angiogenesis. Chronic inflammatory diseases such as arthritis; dermatological disease such as psoriasis, telangiectasis, pyogenic granuloma, seborrheic dermatitis and acne are also angiogenesis-dependent.

In particular, angiogenesis is essential to metastasis and growth of cancer. (D'Amato RJ and Adamis AP, *Ophthalmol* 102, 1261-1262, 1995; Arbiser JL, *J Am Acad Derm* 34, 486-497, 1996; O'Brien K.D. et al. *Circulation* 93, 672-682, 1996; Hanahan D and Folkman J, *Cell* 86, 353-364, 1996) New blood vessels provide not only nutrients and oxygen to fast-growing cancer cells, but also ways of entering the blood stream resulting in metastasis (Polverini P.J., *Critical Reviews in Oral Biology*, 6, 230-247, 1995). Currently, a large variety of chemotherapy and immunotherapy are applied in the treatment of cancer, but the efficacy of the therapies is limited and nothing can successfully extend the life of cancer patients, due to the lack of anti-metastasis effects.

Arthritis, a well-known inflammatory disease, is initiated as an autoimmune disease. However, the growth of vascular endothelial cell in the synovial cavity is activated by the inflammatory cytokines, which finally destroyed cartilage in the articulation. (Kocb AE, Polverini PJ and Lcibovich SJ, *Arth Rheum* 29, 471-479,1986; Stupack DG, Storgard CM and Cheresh DA, *Braz J Med Biol Rcs* 32, 578-581, 1999; Koch AE, *Arthritis Rheum* 41, 951-962, 1998)

Many people are losing their eyesight all over the world because of various ocular diseases. Many patients became blindness due to the infiltration of the capillary blood cells into the vitreous humor (Jeffrey MI and Takayuki A, *J Clin Invest* 103, 1231-1236, 1999).

Psoriasis is caused by extremely active proliferation of skin cells. Fast-growing cells requires sufficient blood supply, and angiogenesis is abnormally induced in psoriasis (Folkman J., *J Invest Dermatol* 59, 40-48, 1972).

As mentioned above, angiogenesis is closely related to initiation and progression of many diseases. Therefore, inhibitors of angiogenesis can be the good candidates for the treatment of those diseases. Many efforts have been made toward the development of angiogenesis inhibitors in order to prevent and/or treat those diseases.

Since the individual cells that make up even a single tumor vessel vary widely, the effectiveness of cancer treatments can be improved with various types of anti-angiogenic therapy. It is desirable to prepare a cocktail of several angiogenesis inhibitors for optimal anti-angiogenic therapy.

One of the major events for inducing angiogenesis is a breakdown of the extracellular matrix before the formation of the capillary blood vessels.

The most important enzyme of matrix degradation is matrix metalloproteinase (MMP), a family of over 20 enzymes. MMPs are endopeptidase, which degrade or proteolyze the components of the extracellular matrix such as collagen, proteoglycan, and gelatin, and are classified into four groups: collagenase, gelatinase, stromelysin, and membrane-type MMP. Many enzymes in the MMP family have substrate specificity. The expression of MMP is induced under various physiological circumstances when remodeling of an extracellular matrix is required. (Curry TE Jr, Osteen KG, *Biol Repord* 64, 1285-1296, 2001; Damjanovske S, Amano T, Li Q, Ueda S, Shi YB, Ishizuya-Oka A, *Ann NY Acad Sci* 926, 180-191, 2000; Ravanti L, Kahari VM, *Int J Mol Med* 6, 391-407 2000)

Increased expression or activation of MMPs is observed in many pathological states, such as atherosclerosis, Alzheimer's disease, skin aging, wrinkle, arthritis, corneal ulcer, proteinuria, abdominal aortic aneurysm, regressive cartilage loss, myelinated nerve loss, liver fibrosis, nephrogromerula disease, germinal membrane ruptures, inflammatory bowel disease, gingivitis/ periodontitis, senile macular degeneration, retinopathy, Sjogren syndrome, myopia, rejection of cornea implantation, angiogenesis and cancer metastasis. (Woessner Jr., *Annals NY Acad Sci*, 732, 11-21, 1994; Warner *et al.*, *Am J Pathol,* 158, 2139-44, 2001; Stetler-Stevenson, *Surg Oncol Clin N Am*, 10, 383-92, 2001)

For example, stromelysins are known to be the major enzyme for disruption of cartilage (Murphy, G. et al., *Biochem J*, 248, 265-268, 1987). Collagenases, gelatinases and stromelysins are responsible for the degradation of the extracellular matrix in many retinopathies (Bruns, F.R. et al.,

Invest Opthalmol and Visual Sci, 32, 1569-1575, 1989). Collagenases and stromelysins are identified in fibroblast from gingiva in inflammation, and the activity of the enzyme is dependent on the degree of inflammation (Beeley, N.R.A. et al., *supra*; Overall, C.M. et al., *J Periodontal Res*, 22, 81-88, 1987).

Recent reports have also shown that MMP-1 activity is highly induced in Alzheimer's disease, and MMP-1 and MMP-3 are involved in the pathophysiology of the disease. (Leake A, Morris CM, & Whateley, *J Neurosci Lett* 291, 201-3, 2000; Yoshiyama Y, Asahina M, & Hattori T, *Acta Neuropathol (berl)*, 99, 91-5, 2000)

MMPs are also responsible in solar UV radiation-induced skin damage, affecting skin tone and resiliency leading to premature aging. The symptoms of which include leathery texture, wrinkles, mottled pigmentation, laxity and sallowness. Therefore, MMP inhibitors could be included in cosmetics for anti-photoaging or anti-wrinkle agent (Hase T, Shinata K, Murase T, Tokimitsu I, Hattori M, Takimoto R, Tsuboi R and Ogawa H, *Br J Dermatol* 142, 267-273, 2000; Fisher GJ, Talwar HS, Lin J, Voorhees JJ, *Photochem Photobiol* 69, 154-157, 1999).

Since inhibitors for MMP and angiogenesis can be applied for treatment of many diseases, development of angiogenesis inhibitor as new drugs is expected. Desirable inhibitors should not have toxic or adverse effect with good patient compliance because inhibitors need to be administered for a long time.

SUMMARY OF THE INVENTION

The inventors have found that Melissa leaf extract exerts the following

action: Inhibition of angiogenesis, inhibition of the proteolytic activity of matrix metalloproteinase

Accordingly, the present invention provides an anti-angiogenic composition comprising Melissa leaf extract as active ingredient with or without other active ingredients.

More specifically, the present invention provides an anti-angiogenic composition for pharmaceutical or dietetic use.

Thus, the composition of the present invention can be used for the treatment or prevention of diseases derived from angiogenesis

Further, the present invention provides an MMP-inhibitory composition comprising Melissa leaf extract as active ingredient with or without other active ingredients.

More specifically, the present invention provides an MMP-inhibitory composition for pharmaceutical, dietetic, or cosmetic use.

Thus, the composition of the present invention can be used for the treatment or prevention of diseases derived from MMP.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a picture showing the formation of tube by human umbilical vein endothelial cells (HUVEC) grown in Matrigel.

Figure 2 is a picture showing HUVEC treated with 25 μg/ml of Melissa leaf extract.

Figure 3 is a graph of effect of Melissa leaf extract on angiogenesis in mouse Matrigel model.

Figure 4 is a graph showing the inhibition of angiogenesis by oral administration of Melissa leaf extract in mouse Matrigel model.

Figure 5 is a picture showing anti-angiogenic effect of Melissa leaf extract in chorioallantoic membrane assays (a: control, b: treated).

Figure 6 is a graph showing inhibition of MMP-1 activity by Melissa leaf extract.

Figure 7 is a graph showing inhibition of MMP-2 activity by Melissa leaf extract.

Figure 8 is a graph showing inhibition of MMP-9 activity by Melissa leaf extract.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

Hereinafter, the present invention will be explained in detail.

The inventors have found that Melissa leaf extract of this invention inhibits angiogenesis not only in tube formation assay, but also in CAM assay and mouse Matrigel model. Melissa leaf extract also inhibits angiogenesis when it is orally administered.

The tube formation assay is an *in vitro* experimental method that is closely related to *in vivo* efficacy, and this assay investigates the effect on the migration and differentiation of human endothelial cell forming microvascular network. While the CAM assay is an *in vivo* assay using fertilized eggs, angiogénesis can be quantitatively measured in mouse Matrigel model.

Further, the inventors have found that Melissa leaf extract inhibited MMP, a family of essential enzymes for angiogenesis and cancer metastasis. When

the effect of Melissa leaf extract on MMPs is investigated with MMP-1, MMP-2, and MMP-9, it drastically inhibits activity of all three enzymes. The inhibitory effect of Melissa leaf extract on MMPs is not, however, limited to these three enzymes.

It is therefore clear that the composition comprising Melissa leaf extract of the present invention is used as an anti-angiogenic agent for the treatment or prevention of angiogenesis-dependent diseases, for pharmaceutical or dietetic use.

It is therefore also clear that the composition comprising Melissa leaf extract of the present invention is used as an MMP-inhibitory agent for the treatment or prevention of MMP-related diseases, for pharmaceutical, dietetic or cosmetic use.

Melissa leaf extract used in the present invention can be purchased or prepared with conventional methods. Commercially available Melissa leaf extract can also be used. The example of the conventional extraction method is as follows.

In brief, 10 to 20 L of an aqueous alcohol (for example, methanol, ethanol, butanol, etc.) or acetone is added to 1 kg of dried *Melissa* leaves. The mixture is allowed to extract at a temperature ranging from 60 to 80℃, for a period ranging from 30 min to 2 hours. The extraction process may be repeated 2 to 3 times with other solvents (chloroform, ethyl acetate, ketone, etc.). The resulting extract is concentrated to obtain a Melissa leaf extract.

As mentioned above, Melissa leaf extract of the present invention has inhibitory effects on angiogenesis and MMP activity. While MMPs are enzymes responsible for angiogenesis, anti-angiogenic activity of Melissa leaf

extract is not limited to MMP inhibitory activity. That is, though MMPs are one of the factors for inducing angiogenesis, Melissa leaf extract can inhibit other factors of angiogenesis. Furthermore, the inhibitory of activity on MMP of Melissa leaf extract are not limited to inhibition of angiogenesis.

The composition of the present invention comprising Melissa leaf extract may also comprise more than one component of other angiogenesis inhibitors, such as ticlopidine, glucosamine (2-amino-2-deoxy-D-gucopyranose), horse chestnut extract and *Ginkgo biloba* extract for the prevention and/or treatment of angiogenesis- and MMP-dependent diseases. We have previously reported that angiogenesis is inhibited by commercially available pharmaceutical composition such as horse chestnut extract (KR10-2001-66246), glucosamine and its salt (KR-10-2001-18675), *Ginkgo biloba* extract (KR10-2000-45265) and ticlopidine (KR10-2000-43589).

These commercially available drugs can be co-treated with Melissa leaf extract of present invention to potentate the effect of the composition.

Specifically, combined treatment of Melissa leaf extract with *Ginkgo biloba* extract or ticlopidine can be used as metastasis inhibitors.

The composition of the present invention comprising Melissa leaf extract may also comprise more than one component of other anti-cancer, anti-inflammatory and anti-aging agents such as Glycyrrhiza glabra, Cinnamonum cassia, Sophora japonica, Atractylodes japonica, Atracylodes lancea, Artemisia capillaris, Morus alba, Houttuynia cordata, Lonicera japonica, Inula japonica, Inula britannica, Paeonia albiflora, Paeonia japonica, Paeonia obovata, Curcuma domestica, Curcuma longa, Saururus chinensis, Vaccinium myrtillus, Rubus spp., Melilotus officinalis, Agelica gigantis, Salvia officinalis, Salvia

miltorrhiza, Liriope platyphylla, Zingiber officinalis, Ulmus cavidiana, Ulmus macrocarpa, Camellia japonica and Vitis vinifera. Above compositions can be added to drugs, quasi-drugs, foods or beverages used for anti-angiogenic purpose.

The anti-angiogenic activity of above component is also confirmed by tube formation of HUVEC as previously mentioned. The inhibition of tube formation by 50 μg/ml of each composition was 30-100% as compared with non-treated control HUVEC. For example, percent inhibition was 100% for Cinnamonum cassia, 51.7% for Atractylodes japonica, 53% for Artemisia capillaris, 53% for Morus alba, 40% for Vaccimium myrtillus, 30% for Houttuynia cordata, and 38% for Paeonia japonica.

A composition comprising Melissa leaf extract can also comprises more than one kind of diluent including dextrose, maltodextrin, saline, buffered saline, water, glycerol, and ethanol, but the diluent is not limited. Appropriate diluents are listed in the written text of Remington's Pharmaceutical Science (Mack Publishing co, Easton PA).

Formulations containing Melissa leaf extract may be prepared in any form. The formulation can be prepared as injectable preparation (true solution, suspension, or emulsion) and preferably in oral dosage form (tablet, capsule, soft capsule, aqueous medicine, pill, granule) and topical preparation (ointment, patch, spray, solution, and the like).

The composition comprising Melissa leaf extract of the present invention can be administered by various routes. The route of administration includes oral, intravenous, intraperitoneal, subcutaneous, intramuscular, intra-arterial, transdermal, rectal, nasal, ocular, and topical application.

The composition comprising Melissa leaf extract of the present invention may be applied differently according to the diseases and route of administration. It should be understood that the amount of active ingredient has to be determined with various factors. These factors include the severity of the patient's symptoms, other co-administered drugs (e.g., chemotherapeutic agents), age, sex, body weight of the individual patient, food, dosing time, the chosen route of administration, and the ratio of the composition.

A daily dose of Melissa leaf extract is preferable from about 5 mg to 2 g, most preferably 10 to 1000 mg. In general, 0.1 to 200 mg/kg of Melissa leaf extract can be administrated in a single dose or 2-3 divided doses per day.

The cosmetic composition comprising Melissa leaf extract of the present invention can be used for photoaging or wrinkle treatment.

The following examples are intended to further illustrate the present inventions. However, these examples are shown only for better understanding the present invention without limiting its scope.

<EXAMPLE 1>

Melissa leaf extract was purchased from Emil Flachsmann AG and used in the following examples.

<TEST 1> Effect of Melissa leaf extract on tube formation of HUVEC

The effect of Melissa leaf extract on angiogenesis was investigated *in vitro* with human endothelial cells.

To perform the tube formation assay, human umbilical vein endothelial cells (HUVEC) were isolated from freshly obtained cords after cesarean

section. Cells were cultured and identified by immunocytochemical staining with anti-Factor VIII antibody. HUVEC grown with Matrigel (BD Bioscience, Bedford, MA, USA), were treated with 25 μg/ml of the above Melissa leaf extract (Emil Flachsmann AG) of the EXAMPLE 1, and further incubated at 37℃ for 8-16 hrs. As a control, the procedure was repeated without Melissa leaf extract.

Fig. 1 shows that a tubular network is formed as a process of neovascularization, when they are grown on Matrigel. However, the microvascular network of HUVEC on Matrigel was disconnected by Melissa leaf extract as shown in Fig. 2.

Fig. 2 is the HUVEC grown on Matrigel treated with 25 μg/ml of Melissa leaf extract, which shows that the microvascular network was disconnected.

The area of the tube was determined by the image analysis program Image-Pro Plus® (Media Cybernetics, USA), and summarized in Table 1. Tube formation after treatment of Melissa leaf extract was inhibited by about 66% as compared with the untreated control.

(Table 1)

	Area of tube	%
Control	11.56	100
Melissa leaf extract	3.92	34

<TEST 2> Animal experiment for angiogenesis (mouse Matrigel model)

The anti-angiogenic activity of *Melissa* leaf extract was quantitatively investigated in mouse Matrigel model.

A 0.4 ml portion of Matrigel mixed with each of 50 ng/ml of basic fibroblast growth factor (bFGF) and 50 units/ml of heparin was implanted to C57BL/6 female mice of 6 to 8 week-old by subcutaneous injection. After 3-5 days, Matrigel was removed from excised skin of each mouse, the level of hemoglobin (Hb) in the Matrigel was measured with a Drabkin kit (Sigma Chemical Co., St. Louise, MI, USA, Cat. No. 525), a reagent for determination of total hemoglobin in blood.

The same experiment was done with Matrigel cotaining Melissa leaf extract (0.5 mg) of the EXAMPLE 1, and hemoglobin content of the treated group was compared with that of the control group. As shown in Fig. 3 and Table 2, the hemoglobin content of the treated group was remarkably reduced as compared with that of the control group. Therefore, angiogenesis was inhibited by about 99%.

(Table 2)

	Hemoglobin (g/dL)
Control	453 ± 446
Melissa leaf extract	3 ± 7

In order to test the activity of orally administered Melissa leaf extract on angiogenesis, the following experiment was undertaken.

A 0.4 ml portion of Matrigel containing 50 ng/ml of basic fibroblast growth factor (bFGF) and 50 units/ml of heparin was implanted by subcutaneous injection, and 0.6 mg of Melissa leaf extract per mouse was orally administered twice a day for 4 days. At day 5, the Matrigel was removed and the amount of hemoglobin in the Matrigel was determined.

As shown in Fig. 4 and Table 3, the Melissa leaf extract-treated group showed a lower level of hemoglobin in Matrigel, about 71% of that of the

control group. Therefore, Melissa leaf extract also showed anti-angiogenic activity when it was administered orally.

(Table 3)

	Hemoglobin (g/dL)
Control	109 ± 198
Melissa leaf extract	32 ± 38

<TEST 3> Angiogenesis assay with chorioallantoic membrane assays (CAM assay)

Fertilized chicken eggs were kept in a humidified incubator at 37℃. After incubation for three days, 2-3 ml of albumin was aspirated from the eggs with a syringe of 26-gauge needle and the egg was sealed with transparent tape. A window of a small hole was drilled at the end of the eggs. Two days later, an aliquot of 50 μg of Melissa leaf extract dissolved in 15 μl of saline was applied to sterile Thermanox discs (Miles Scientific) and allowed to air dry. The discs were applied to the chorioallantoic membrane surface through the window and covered with transparent adhesive tape. The embryos were incubated for further three days at 37℃ in a humidified incubator. An appropriate volume of lipid emulsion was injected into the embryo chorioallantois using a 26-gauge needle so that the vascular network of the chorioallantoic membrane stood out against the white lipid background. As a control, 15 μl of physiological saline was loaded to a disc instead of Melissa leaf extract following the same procedure as mentioned above. The resulting blood vessels were observed and compared with treated eggs.

In the control group (n=20), capillary vessel formation was not affected in 90% of the embryo (Fig. 5a), while the inhibition of vessel formation in the

disc (brighter part of the picture) treated with Melissa leaf extract was significant and the inhibition of the blood vessel formation of the chorioallantois was observed in all the treated eggs (n= 15, 100%, Fig. 5b).

<TEST 4> Effect of combined treatment of Melissa leaf extract with *Ginkgo biloba* extract on angiogenesis inhibition

The effect of co-treatment of Melissa leaf extract with other composition on angiogenesis was investigated in mouse Matrigel model.

A 0.4 ml portion of Matrigel containing 50 ng/ml of basic fibroblast growth factor (bFGF) and 50 units/ml of heparin was implanted by subcutaneous injection, and 1.0 mg of Melissa leaf extract combined with 0.5 mg of *Ginkgo biloba* extract was orally administered twice per day for 4 days.

Lower dose of combined composition of Melissa leaf extract with Ginkgo biloba extract were also given to mice in another group, which were taken one fifth of the previous amount of combined composition (0.2 mg of Melissa with 0.1 mg of Ginkgo).

The amount of hemoglobin in the Matrigel was determined and the result was compared with the non-treated control group. As summarized in Table 4, the average of total hemoglobin levels in the Matrigel from treated group were about 10-18% of that of the control group. The percent inhibition of angiogenesis by combined treatment was 82-90%, which was greater than the Melissa leaf extract alone.

(Table 4)

Treatment	Hemoglobin (g/dL)	Inhibition (%)
Control	162 ±174	0

Melissa (1.0 mg) + Ginkgo (0.5 mg)	29 ± 28	90
Melissa (0.2 mg) + Ginkgo (0.1 mg)	17 ± 19	82

<TEST 5> Inhibition of cancer metastasis by combined treatment of Melissa leaf extract with other composition

B16BL6 cells ($5x10^4$) were injected into C57BL/6 male mouse of 6 to 7 weeks old through the tail vein. After that, 0.2 ml of water or drug combinations was daily given to mice by oral administration for 3 weeks. Three weeks after injection, the mice were sacrificed and the number of tumor colonies on the surface of lungs was counted under microscope. The average number of melanoma colonies in lungs from mice of treated group was less than that from control mice. The percent inhibition of metastasis is 37-38% in single drug treated groups, while it was reduced to 50-54% in combined treatment groups (Table 5).

That is, the combined treatment of Melissa leaf extract with other angiogenesis inhibitor is more potent than Melissa leaf alone.

(Table 5)

Preparation	Colonies in lung	Inhibition (%)
Control	133 ± 39	0
Melissa leaf extract	84 ± 24	37
Ginkgo biloba extract	82 ± 21	38
Ticlopidine	83 ± 20	38
Melissa + Ginkgo	61 ± 15	54
Melissa + ticlopidine	67 ± 17	50

<EXAMPLE 2>

(1) Preparation of MMP

MMP-1, MMP-2, and MMP-9 were cloned and prepared from insect cells (Sf21 insect cell) using Baculovirus system.

MMP-2 cDNA (GENEBANK No. XM_048244) was cloned to a pBlueBac4.5 transfer vector (Invitrogen, Cat no. V1995-20), and then transfected to Sf21 cells with Bac-N-Blue Transfection Kit (Invitrogen, Cat no. K855-01). Sf21 cells were incubated with TNM-FH (Sigma, St. Louis, MO, U.S.A) media containing 10% fetal bovine serum at 27℃, then harvested and re-suspended at a concentration of 10^7 cell/ml. The cell suspension was incubated with a virus containing the cloned gene for 1 hr at room temperature. Infected Sf21 cells were grown for 72 hrs and the medium was recovered. MMP-2 was purified with a gelatin-sepharose affinity column from the recovered medium.

MMP-1 (GENEBANK NO. XM_040735) and MMP-9 (GENEBANK NO. XM_009491) were prepared from corresponding genes as previously described. MMP-1 was purified with SP-sepharose, and MMP-9 was purified by gelatin-sepharose affinity chromatography.

(2) Inhibition of MMP activity

In order to investigate MMP inhibition by Melissa leaf extract, MMP enzyme activity was assayed by a spectrofluorometric method (Perkin-Elmer LS50B).

Purified MMP-1, MMP-2, and MMP-9 were used after activation with 1 mM APMA before assay.

The substrate for MMP-1 and MMP-9 was 2,4-dinitrophenyl-Pro-Leu-Ala-Leu-Trp-Ala-Arg (Sequence No. 1), and Mca-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH_2 (Sequence No. 2 :BACHEM, Cat. No. M-1895) was used as a

substrate for MMP-2.

As a control, 10 nM MMP-1 and 1 μM substrate (Sequence No. 1) were mixed in 2 ml of reaction buffer (50 mM Tricine (pH 7.5), 10 mM $CaCl_2$, 200 mM NaCl) in a 2 ml cuvette. Fluorescence intensity was measured every 2 min for 20 min at room temperature with a spectrofluorometer under an excitation wavelength of 280 nm and an emission wavelength of 360 nm.

Melissa leaf extract (25 μg/ml) dissolved in water and 10 nM MMP-1 was added to a reaction buffer containing a substrate, and fluorescence intensity was measured in the same manner.

Activity for MMP-2 or MMP-9 was also assayed, and fluorescence intensity was measured as previously mentioned.

Figures 6, 7 and 8 are diagrams of activity of MMP-1, MMP-2, and MMP-9. As shown in Fig. 6, 57% of MMP-1 activity was inhibited by Melissa leaf extract. The inhibition of MMP-2 and MMP-9 by Melissa leaf extract was 71% (Fig. 7) and 73% (Fig. 8), respectively.

As previously mentioned, Melissa leaf extract of the present invention inhibits angiogenesis and matrix metalloproteinase activity. Based on that, Melissa leaf extract can be used as a new composition for treatment or prevention of angiogenesis- and/or MMP-dependent diseases.

WHAT IS CLAIMED IS:

1. A composition comprising Melissa leaf extract for inhibiting angiogenesis.

2. The composition according to claim 1, wherein the composition additionally comprises at least one of ingredient selected from the group of *Ginkgo biloba* extract, ticlopidine, glucosamine and horse chestnut extract, Glycyrrhiza glabra, Cinnamonum cassia, Sophora japonica, Atractylodes japonica, Atracylodes lancea, Artemisia capillaris, Morus alba, Houttuynia cordata, Lonicera japonica, Inula japonica, Inula britannica, Paeonia albiflora, Paeonia japonica, Paeonia obovata, Curcuma domestica, Curcuma longa, Saururus chinensis, Vaccinium myrtillus, Rubus spp., Melilotus officinalis, Agelica gigantis, Salvia officinalis, Salvia miltorrhiza, Liriope platyphylla, Zingiber officinalis, Ulmus cavidiana, Ulmus macrocarpa, Camellia japonica and Vitis vinifera.

3. The composition according to claim 1 or 2, wherein the composition is pharmaceutical composition for angiogenesis inhibition.

4. The composition according to claim 1 or 2, wherein the composition is food composition for angiogenesis inhibition.

5. The composition according to claim 1 or 2, wherein the composition is used for prevention and/or treatment of at least one of diseases selected from the group consisting of cancer metastasis, angioma, angiofibroma, diabetic retinopathy, premature infant's retinopathy, neovascular glaucoma, corneal disease induced by angiogenesis, involutional macula, macular degeneration, pterygium, retinal degeneration, retrolental fibroplasias, granular conjunctivitis, psoriasis, telangiectasis, pyogenic granuloma,

seborrheic dermatitis, acne, or arthritis.

6. A composition comprising Melissa leaf extract for inhibiting matrix metalloproteinase activity.

7. The composition according to claim 6, wherein the composition additionally comprises at least one of ingredient selected from the group of *Ginkgo biloba* extract, ticlopidine, glucosamine and horse chestnut extract, Glycyrrhiza glabra, Cinnamonum cassia, Sophora japonica, Atractylodes japonica, Atracylodes lancea, Artemisia capillaris, Morus alba, Houttuynia cordata, Lonicera japonica, Inula japonica, Inula britannica, Paeonia albiflora, Paeonia japonica, Paeonia obovata, Curcuma domestica, Curcuma longa, Saururus chinensis, Vaccinium myrtillus, Rubus spp., Melilotus officinalis, Agelica gigantis, Salvia officinalis, Salvia miltorrhiza, Liriope platyphylla, Zingiber officinalis, Ulmus cavidiana, Ulmus macrocarpa, Camellia japonica and Vitis vinifera.

8. The composition according to claim 6 or 7, pharmaceutical composition for inhibiting matrix metalloproteinase activity.

9. The composition according to claim 6 or 7, food composition for inhibiting matrix metalloproteinase activity.

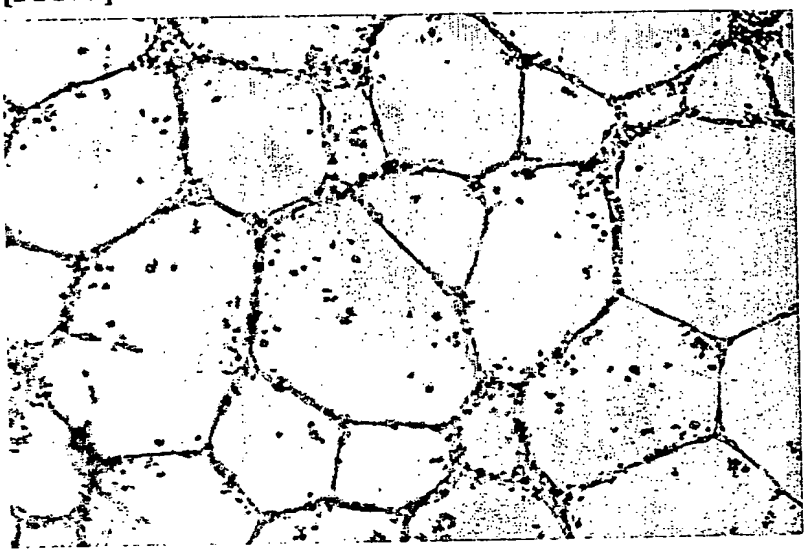
10. The composition according to claim 6 or 7, cosmetic composition for inhibiting matrix metalloproteinase activity.

11. The composition according to claim 6 or 7, wherein the composition is used for treatment of at least one of diseases selected from the group consisting of cancer metastasis, atherosclerosis, restenosis, MMP-dependent osteopathy, inflammation of the central nervous system, Alzheimer's disease, skin aging, corneal ulcer, synechia, bone disease,

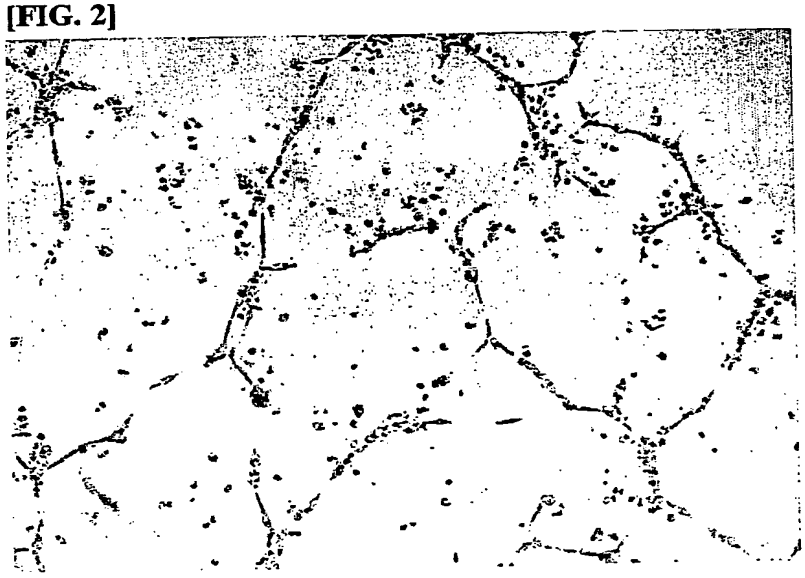
proteinuria, abdominal aortic aneurysm, regressive cartilage loss, myelinated nerve loss, liver fibrosis, nephrogromerula disease, germinal membrane rupture, inflammatory bowel disease, gingivitis/periodontitis, senile macular degeneration, diabetic retinopathy, proliferate vitreous body retinopathy, immature retinopathy, eye inflammation, conical cornea, Sjogren syndrome, myopia, eye tumor, rejection in cornea implantation, rheumatoid arthritis, arthritis or septic arthritis.

[DRAWING]

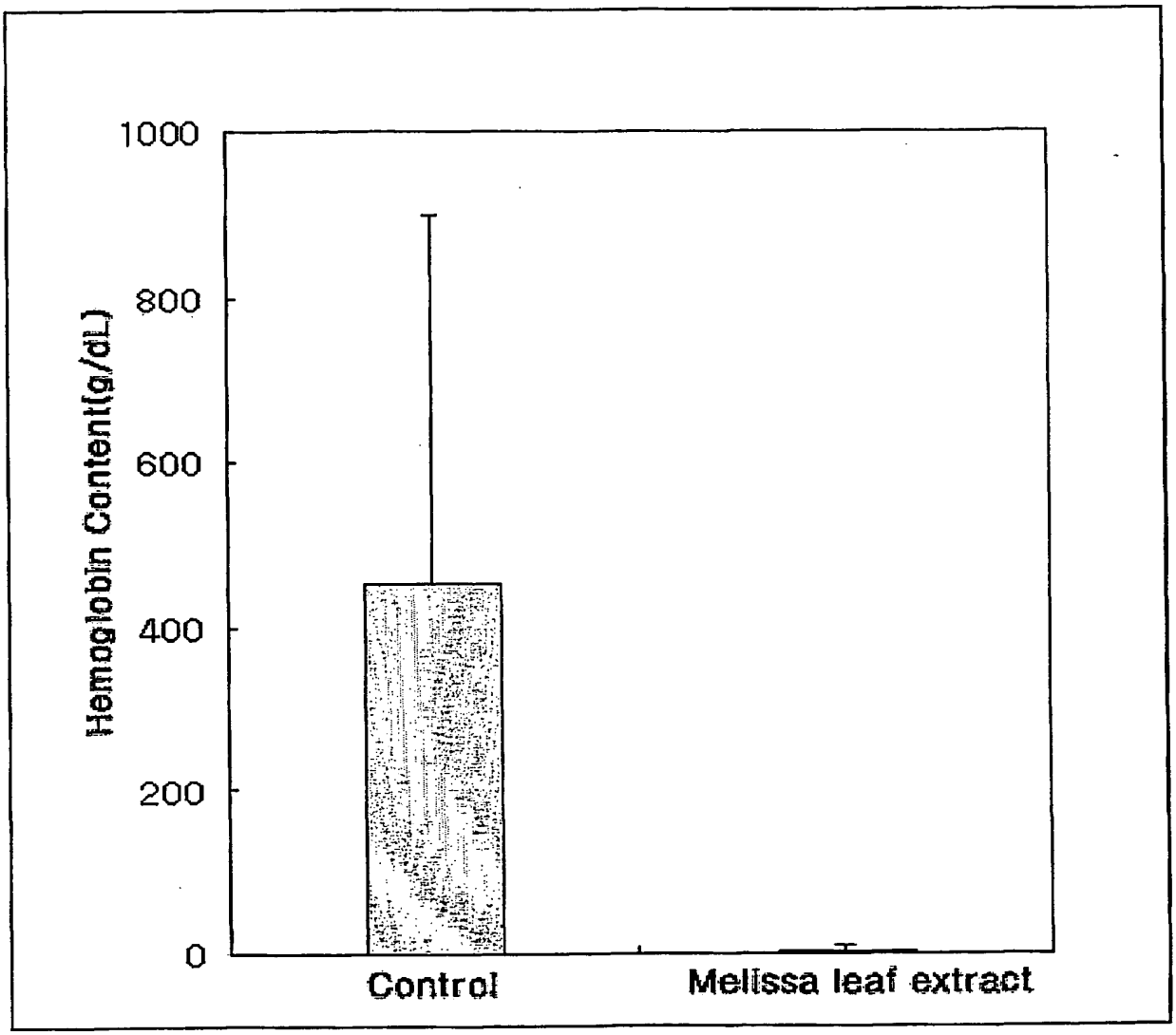
[FIG. 1]



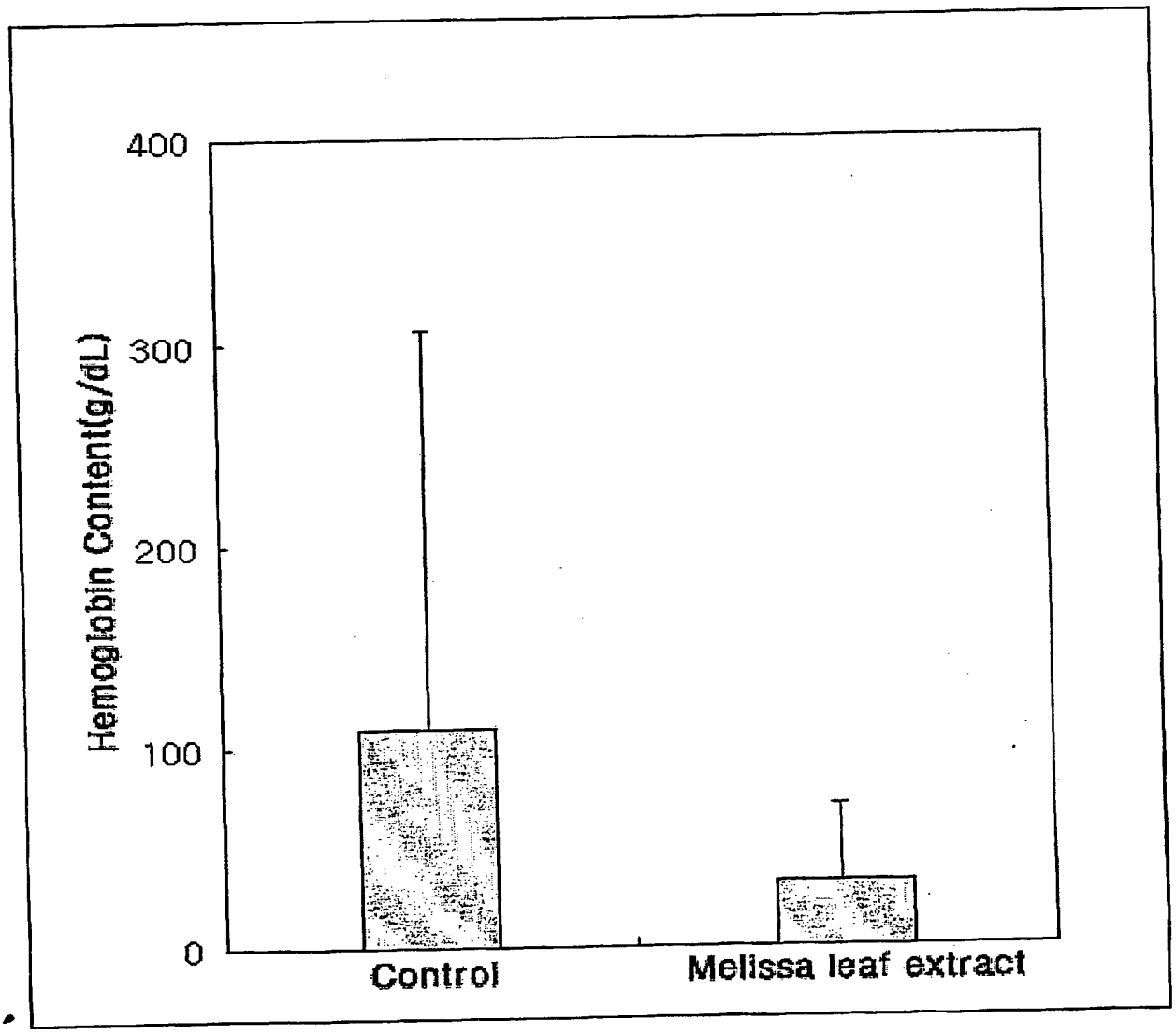
[FIG. 2]



[FIG. 3]



[FIG. 4]



[FIG. 5]

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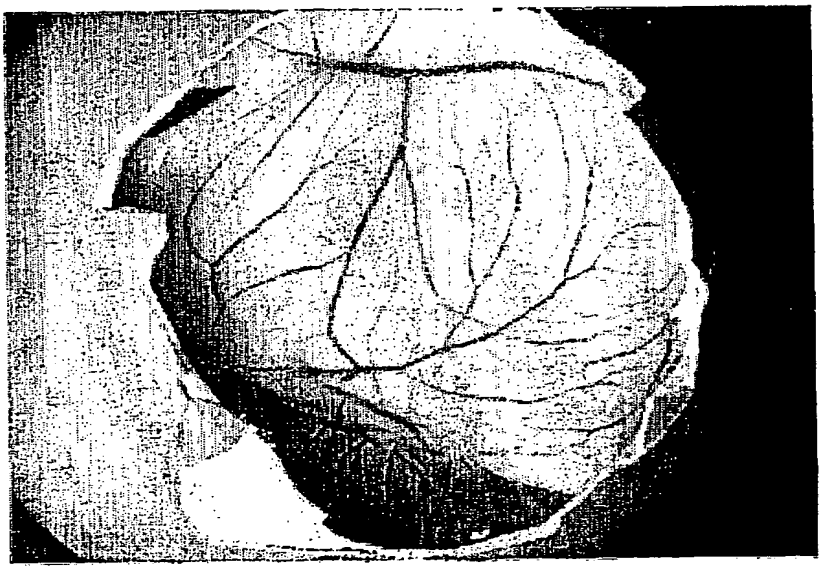

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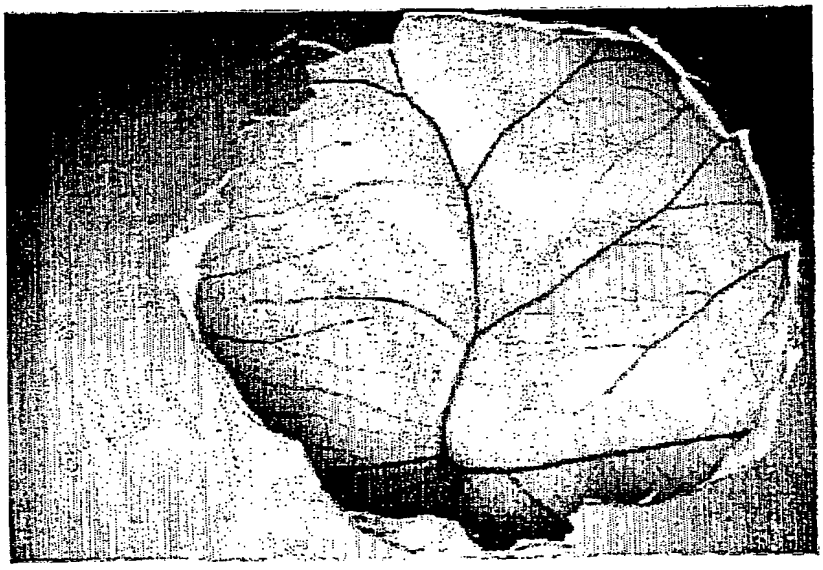
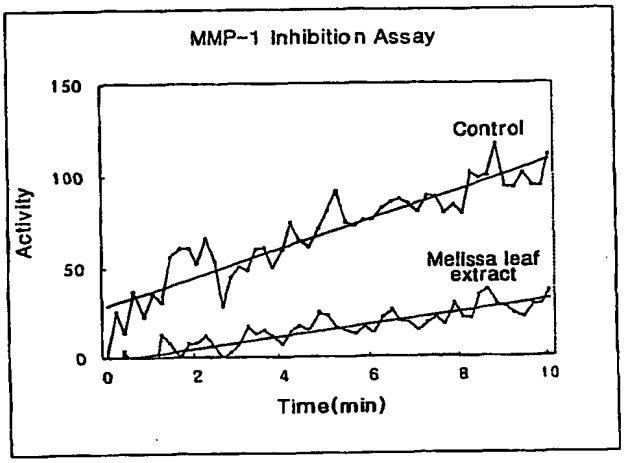
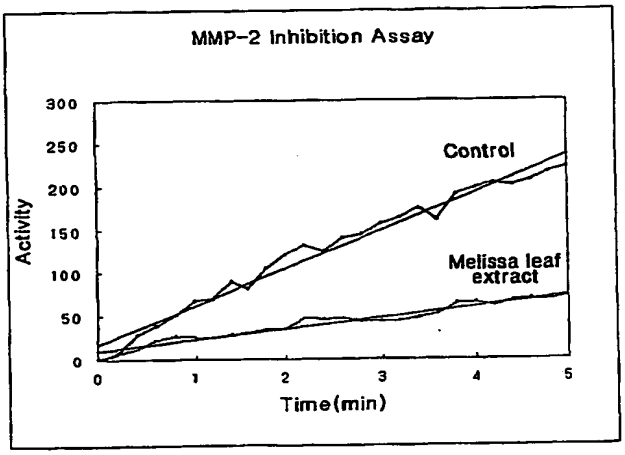


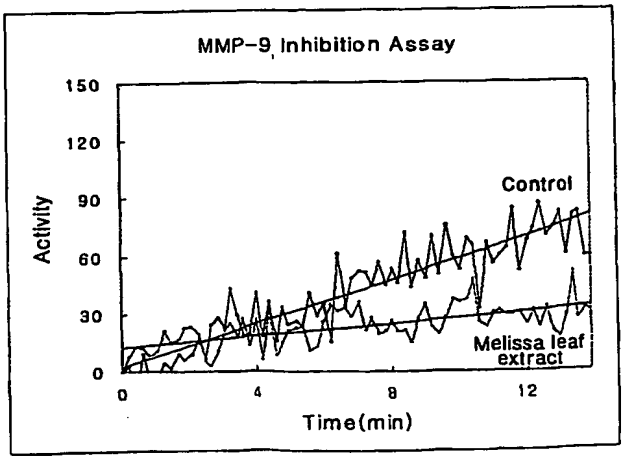
[FIG. 6]



[FIG. 7]



[FIG. 8]



<110> ANGIOLAB, INC.

<120> COMPOSITION COMPRISING MELISSA LEAF EXTRACT FOR ANTI-ANGIOGENIC AND MATRIX METALLOPROTEINASE INHIBITORY ACTIVITY

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<151> 2000-12-12

<150> KR 2001-8470
<151> 2001-02-20

<150> KR 2001-77392
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Pro Leu Gly Leu Ala Arg
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR01/02148

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 A61K 35/78

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7 A61K 35/78

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

KOREAN PATENTS AND APPLICATIONS FOR INVENTIONS SINCE 1975
JAPANESE PATENTS AND APPLICATIONS FOR INVENTIONS SINCE 1975

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN ON LINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP 11-49693 (FOREST GENETICS RESEARCH INSTITUTE), 23 FEB. 1999 SEE THE WHOLE PAGES	1
A	J. PHARM PHARMACOL 1986 NOV;38(11), 791-794	6

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
08 APRIL 2002 (08.04.2002)	09 APRIL 2002 (09.04.2002)

Name and mailing address of the ISA/KR	Authorized officer
Korean Intellectual Property Office Government Complex-Daejeon, 920 Dunsan-dong, Seo-gu, Daejeon Metropolitan City 302-701, Republic of Korea Facsimile No. 82-42-472-7140	CHO, Hee Won Telephone No. 82-42-481-5607

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR01/02148

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 11-49693	23.02.1999.	NONE	

Form PCT/ISA/210 (patent family annex) (July 1998)